42. (New) The retroviral vector particle according to claim 24, wherein the SA site is derived from HIV.

43. (New) The retroviral vector particle according to claim 24, wherein the SD site is derived from MLV.

REMARKS

Pending Claims

Claims 24-41 have been amended. New claims 42-43 have been added. Accordingly, claims 24-43 are pending in this application.

No new matter has been added by this amendment. Applicants submit the amended claims are supported by the specification as filed, for example:

Claim 24: An intron flanked by splice donor (SD) and splice acceptor (SA) sites derived from different retroviruses is supported by the specification, for example on pages 5, 7, and 13.

Page 5, line 13:

> " ...via recognition of a hybrid MLV/HLV intron..."

Page 7, lines 13-15:

> "The preferred method described herein for achieving a Rev-dependent intron in an MLV-based vector is to use the MLV splice donor site and the HIV envelope gene 3' splice site."

Page 13, lines 5-17:

> "This is the first description of the incorporation of an extended HIV-1 RRE element combined with an HIV-1 splice acceptor site into an MLV vector. ... The components of the intron in the present invention are preferably derived from MLV and HIV."

Claim 42: The "SA site" derived from HIV is recited on page 7, line 15.

Page 7, line 15:

> "...the HIV envelope gene 3' splice site."

Claim 43: The "SD site" derived from MLV is recited on page 7, line 14-15.

Page 7, lines 14-15

" ...the MLV splice donor site..."

Examiner's Enablement Rejection:

The Examiner has rejected claim 28 under 35 U.S.C. 112, first paragraph, as being non-enabled by the specification, asserting that a retroviral particle encoding a nucleotide sequence of interest is enabled, but not a retroviral particle that encodes a therapeutic gene. Applicant respectfully traverses this rejection.

The Examiner asserts that the term "therapeutic gene" recited in claim 28 is a statement of intended use in gene therapy, and as such, is not commensurate in scope with the disclosure. In support of this rejection, the Examiner states that gene therapy is highly experimental with no definite success to date, citing Anderson, 1998, *Nature* 392:25-30.

Applicant respectfully disagrees with the Examiner's reasoning and cites the following publications that point to the success of gene therapy with retroviral vectors. See, for example, Demaison et al., 2002, *Hum. Gene Ther.* 13:803-13; Hacein-Bey-Abina et al., 2002, *N. Engl. J. Med.* 346:1241-3; Cavazzana-Calvo et al., 2000, *Science* 288:627-29; Kordower et al., 2000, *Science* 290:767-73; and Journal of Gene Medicine (http://www.wiley.co.uk/genetherapy/clinical).

French researchers have reported what some experts call "the first true gene therapy success", the successful treatment of two infant boys for a rare, inherited immune system disorder (Cavazzana-Calvo et al., *Science*, 2000 Apr 28;288(5466):627-9). The boys were suffering from what is known as a human severe combined immunodeficiency (SCID) X1, which leaves them without any effective immune protection. Patients usually live out their short lives in a sterile environment, hence the term "bubble boy", because any infection would overwhelm them. Dr Alain Fischer of the Necker Hospital in Paris and colleagues managed to fully restore the immune system of two infant boys suffering from X-SCID using gene therapy (Cavazzana-Calvo et al., *Science*, 2000 Apr 28;288(5466):627-9). Severe combined immunodeficiency-X1 (SCID-X1) is an X-linked inherited disorder characterised by an early block in T and natural killer (NK) lymphocyte differentiation. This block is caused by mutations of the gene encoding the gammac cytokine receptor sub-unit of interleukin-2, -4, -7, -9, and -15 receptors, which

participates in the delivery of growth, survival, and differentiation signals to early lymphoid progenitors. After successful preclinical studies, a gene therapy trial in humans for SCID-X1 was initiated, based on the use of complementary DNA containing a defective gammac Moloney retrovirus-derived vector and ex vivo infection of CD34+ cells. After a 10-month follow-up period, gammac transgene-expressing T and NK cells were detected in the gene therapy recipient patients. T, B, and NK cell counts and function, including antigen-specific responses, were comparable to those of age-matched controls. Thus, gene therapy using a retroviral vector to deliver a specific therapeutic gene was able to provide a full correction of disease phenotype and, hence, clinical benefit to the healthy patients for life.

The significance of this work has been recognized by many, including Dr David Nelson of The National Cancer Institute in Bethesda, Maryland who said, "this is the first successful evidence that gene therapy works".

In the words of Dr. William French Anderson (referenced by the Examiner) of the University of California Los Angeles, who performed the first-ever gene therapy experiment in 1990, "Dr. Fischer's work is pivotal, if it had not worked, it would have been extremely depressing. SCID is unique, ... if you can't successfully treat SCID you are not going to be able to treat anything else". Indeed, Dr Anderson was correct in that the gene therapy approach adopted by Dr. Fischer and team has paved the way for further gene therapy applications.

Only one year after the French team's results of a successful gene therapy were published in London, Dr. Adrian Thrasher and his team, using the same approach, but this time a lentivirus pseudotyped retrovirus vector, reported: "A child was cured of a fatal genetic condition, X-SCID, by gene therapy using recombinant retroviral vectors, at Great Ormond Street Hospital, London". Bone marrow cells from the patient (18 month old boy) were collected and then a healthy version of the mutated gene was introduced into the bone marrow cells, *ex vivo*, following transduction with a lentivirus pseudotyped retrovirus vector (isolated from gibbon) in order to replace the mutated gene (details of the mutated gene are described above). Transduced bone marrow cells expressing the healthy gene were then re-introduced into the patient which then differentiated into mature immune cells capable of mounting competent immune response. As a result of gene therapy, the boy is now leading a normal life. (See Demaison et al., 2002, *Hum. Gene Ther.* 13:803-13 and Hacein-Bey-Abina et al., 2002, *N. Engl. J. Med.* 346:1241-3.)

Furthermore, Dr. Adrian Thrasher and team have adopted the same gene therapy method to treat a different illness known as chronic granulomatous disorder (CGD) and the results of this treatment will be published in the near future.

In a further example, researchers at Rush-Presbyterian-St. Luke's Medical Centre, Chicago, and Lausanne, Switzerland, have successfully used gene therapy to reverse the anatomical, cellular changes that occur in the brains of primates with Parkinson's disease. The same team also showed success in preventing the disease from progressing and reversing functional deficits or symptoms associated with the disease in monkeys displaying early signs of Parkinson's disease (Kordower et al., 2000, *Science* 290:767-73). Importantly, the researchers used modified lentiviruses (lenti-GDNF) to deliver the gene glial-derived neurotrophic factor (GDNF) directly to the brain cells of monkeys.

Furthermore, we would like to draw the Examiner's attention to an Internet site which is hosted by the publishers of the Journal of Gene Medicine (http://www.wiley.co.uk/genetherapy/clinical) where the results of clinical trials, using gene therapy to treat different diseases are reported for the benefit of the public. Importantly, retroviral vectors are the most widely used vectors in gene therapy clinical protocols.

Thus, it is evident from the above examples that gene therapy works. These examples also show that retroviruses are the gene therapy vectors of choice. According to Professor Inder Verma of the Salk Institute for Biomedical Studies, La Jolla, California, retroviruses have the advantage that they can infect non-dividing cells and can be designed to infect many different cell types. A further advantage is that retroviruses can be manipulated to avoid the immune system and as a result there has been no evidence of adverse immunological reactions to the retroviruses in laboratory animals or in humans.

Thus the applicant submits that gene therapy works and that a skilled person using the teachings of the present invention would be able to construct a retrovirus particle that comprises a nucleotide sequence encoding a therapeutic gene for use in gene therapy.

Furthermore, while Applicant asserts that the use of vectors carrying a "therapeutic" gene is enabled for gene therapy as discussed above, it should be noted that the claimed vector is additionally enabled for method that do not encompass gene therapy. For example, the claimed vectors are useful for the *in vitro* transduction and infection of a target cell, followed by selection of cells expressing the inserted nucleotide sequence (NS), for example, as recited in claims 40

and 41. In this scenario, the vector carrying the "therapeutic" gene (NS) is considered a therapeutic "tool" for the selection of target cells expressing the "therapeutic" protein, and as such, the specification fulfills the enablement requirement of 35 U.S.C. 112, first paragraph, for the vector of claim 28.

Accordingly, Applicant respectfully asserts that claim 28 is fully enabled by the specification. Removal of this enablement rejection is requested.

Examiner's Indefiniteness Rejection:

The Examiner has rejected claims 24-41 under 35 U.S.C. 112, second paragraph, as being indefinite. Specifically, the Examiner objects to the claim phrases, "genome based on a first retrovirus" and "retrovirus on which the vector particle is based". The claims have been amended to delete these phrases. Removal of the rejection is requested.

Examiner's Indefiniteness Rejection:

The Examiner has rejected claims 24-41 under 35 U.S.C. 112, second paragraph, as indefinite for use of the phrases, "functional portions thereof" and "a functional equivalent thereof". Applicant respectfully traverses these rejections.

A. Functional portion thereof (Claim 24)

The Examiner is requested to refer to the specification, where the "functional portion" of an LTR's U3 and R region is described as a complete promoter region, or fragment thereof, which is responsive to Tat transactivation. See, for example:

In paragraph bridging page 9, lines 26-30, and page 10, lines 1-4:

> "In more detail, TIN vectors are constructed with the following points in mind. Tat acts on the TAR region of R, but also requires sequences in the U3 region to function properly. Certain sections of both U3 and R can be removed while still leaving an effective Tat-responsive element. Deletions in HIV-2 U3 result in promoters with lower basal levels of transcription that still remain responsive to Tat (Brady et al., 1994). Thus, the transcriptional

control is preferably provided by an HIV Tat-inducible promoter comprising the functional portions of both U3 and R from HIV. Alternatively, certain U3 and/or R sequences from other retroviruses, which are responsive to the HIV Tat protein, may be used, for example, U3 from RSV linked to R from HIV. The TIN vector provides a means of preserving the Tat response within the context of a simple retroviral vector, e.g. one based on MLV such that MLV packaging systems may be used."

On page 10, lines 4-8:

"Alternatively, certain U3 and/or R sequences from other retroviruses, which are responsive to the HIV Tat protein, may be used, for example, U3 from RSV linked to R from HIV. The TIN vector provides a means of preserving the Tat response within the context of a simple retroviral vector, e.g. one based on MLV such that MLV packaging systems may be used."

On page 13, lines 28-30:

"In addition, some other promoters, including the U3 regions from HIV-2 and RSV can substitute for the HIV-1 U3 regions to allow Tat transactivation (Liu et al., 1994)."

Secondly, in the context of the present invention, the transcriptional transactivator may be any factor derived from other retroviruses, or from a host cell, that is analogous to Tat, and via a specific interaction with TAR, acts as a transcriptional transactivator. Tat/TAR analogous systems are known in other retroviruses, such as Tax/TxRE in the human T-cell leukemia virus (HTLV), the visna virus Tat protein (Neuveut et al., 1993, *Virology* 197:236-44), human foami virus (HFV) transcriptional transactivator bel1 (Lee et al., 1992, J. Virol. 66:3236-40), and, in particular, MLV, ASLV, SNV, and RSV (see specification, the paragraph bridging pages 15 and 16).

Thus, the term "functional portions thereof" as recited in claim 24 clearly and unambiquously refers to functional portions of the claimed U3 and R regions of the 5'LTR, and is well supported by the specification.

B. Functional equivalent thereof (Claims 26, 27)

The Examiner is requested to refer to the specification, where a "functional equivalent" of a PRE is described as a fragment, a mutated, or otherwise manipulated version of PRE that retains its capacity to act as a nucleus to cytoplasm transport factor. In the context of the present invention, the nucleus to cytoplasm transport factor may be HIV Rev, or any factor derived from a different virus or host cell that has a similar function to HIV Rev, i.e, binds to the RRE and functions as a cytoplasm transport factor. Several such sytems are known. See, for example:

On page 6, lines 23-29:

> "The response element in the retroviral vector genome is chosen according to the specific conditions under which expression of the therapeutic gene is desired. If expression is to be HIV dependent, then a suitable response element is RRE or a functional equivalent which responds to Rev. A functional equivalent of RRE may be for example a portion of RRE or a mutated or otherwise manipulated version of RRE which retains the desired activity."

On page 7, lines 1-8:

> "The transport factor may thus be HIV Rev, rendering expression of the therapeutic gene dependent upon the presence of HIV. The transport factor may alternatively be any factor, originating e.g. from other viruses or from host cells, which is analogous to Rev in that it enables transport to the cytoplasm via a specific interaction with the response element. Systems analogous to the Rev/RRE system are known, for example in

other retroviruses. One such example is the rex/RxRE system in HTLV-1."

Thus, the specification clearly defines the claimed PRE to include elements such as RRE and functional equivalents that respond to a nucleus to cytoplasm factor able to transport an RNA transcript via a specific interaction with an RNA response element. Rev/RRE analogous systems are known in other retroviruses such as the Rex/RxRE system in the human T-cell leukemia virus, as well as other retroviruses (see Coffin, J. Retroviruses, 1997, Chapter 2, Cold Spring Harbor Laboratory Press, USA).

Thus, the term "a functional equivalent thereof" as recited in claims 26 and 27 clearly and unambiquously refers to functional equivalents of the claimed PRE, and is well supported by the specification.

Examiner's Obviousness Rejection:

The Examiner has rejected claims 24-41 under 35 U.S.C. 103(a) as being unpatentable over Lisziewicz (1992, WO92/21750) in view of Hope et al. (1990, *Proc. Natl. Acad. Sci.* 87:7787-91) and Naldini et al. (1996, *Science* 272: 263-67). Applicants respectfully traverse this rejection.

To establish a prima facie case of obviousness, the Examiner must show that 1) the prior art references teach or suggest all of the claimed limitations; 2) there is some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine the teachings; and 3) there is a reasonable expectation of success. MPEP § 2142. For the reasons provided herein, Applicants assert that the Examiner has not met the burden of showing that the prior art references teach or suggest all of the claimed elements.

The cited references do not teach every element of the invention as presently claimed.

The claimed invention recites retroviral vectors that comprise Tat-inducible promoter sequences, polynucleotide response elements (PRE), and nucleotide sequence (NS) capable of being expressed in a target cell. Further, the required location of the Tat inducible sequence is within the HIV U3 and R of the 5'LTR of the retroviral vector. The claims require the NS and

PRE to be within a hybrid intron defined by flanking splice donor (SD) and splice acceptor (SA) sites that are derived from different retroviruses.

In contrast to the Examiner's assertions, the claimed combination of elements is not disclosed or suggested in any of the cited references, alone or in combination.

A. Liesziewicz does not teach or suggest the claimed retroviral vector.

Lisziewicz (WO 92/21750) describes retroviral vectors having a TAT-inducible promoter (HIV), expressible gene, and PRE. High viral titer is achieved by increasing the amount of packageable RNA in the cytoplasm via the Rev/RRE transport element. Liesziewicz does not teach a splicing acceptor (SA) site adjacent to the Rev-responsive element, RRE, nor the hybrid intron required by the pending claims. Although Lisziewicz refers to an "intron" and "intron containing RNA" or "an intron of a foreign gene," Lisziewicz makes no reference to a hybrid intron containing different retroviral SD and SA sites flanking the NS and PRE in the vector. In particular, Lisziewicz does not teach or disclose a retroviral vector producing a DNA provirus that is doubly regulated by the HIV-produced activators, Tat-responsive promoter and PRE, as claimed.

In summary, Lisziewicz fails to disclose or suggest a retroviral vector having the specific combination of TAT regulatory elements within the 5' LTR region and hybrid intron with SD and SA sites from different retroviral sources containing the NS and PRE. These features provide a unique vector that is completely HIV-dependent, lacking "leaky" expression of the contained NS, and lead to undetectable basal levels of expression of the coding sequence in the absence of HIV. See, for example:

Page 5, lines 7-11:

> "Moreover, we show that the inclusion of an extended sequence derived from the HIV env region which contains RRE and the 3' splice acceptor sequence from env produces a new vector pTRAC for which the basal transcription levels are undetectable in the absence of Tat and Rev."

Accordingly, Lisziewicz fails to teach or suggest the claimed invention.

B. The secondary reference, Hope et al., does not cure the deficiencies of Liesziewicz.

Hope et al. does not cure the deficiencies of Liesziewicz; the reference also fails to teach or suggest the claimed doubly regulated HIV-responsive vector containing a hybrid intron. Hope teaches a reporter plasmid (pDM128) derived from the Env region of HIV-1, and containing the reporter gene CAT and PRE in an intron. Although Hope demonstrates the need for a functional PRE for Rev-induced transport and expression of unspliced genes, Hope does not disclose or suggest the claimed combination of elements. In particular, neither Lisziewicz or Hope discloses or suggests the claimed hybrid intron comprising SD and SA sites derived from different retroviral sources.

In summary, Hope discloses a plasmid derived from a viral vector containing a reporter gene and PRE within an intron. However, Hope discloses neither a retroviral vector with the specific regulatory elements in the 5' LTR region, nor the presence of a hybrid intron comprising SD and SA sites from different sources within the vector.

Accordingly, Hope et al. fails to cure the deficiencies of Lisziewicz. Hope et al., either alone or in combination with Lisziewicz, fails to teach or suggest the claimed invention.

C. Naldini et al. does not cure the deficiencies of Liesziewicz or Hope et al.

Naldini et al. discloses an HIV-derived retroviral vector that is capable of integrating into the genome of non-proliferating cells. Construction of this vector is based upon a three-plasmid expression system, utilizing a separate packaging construct, Env-encoding plasmids from VSV or MLV, and a transfer vector. Because it is derived from HIV, the resultant vector inherently contains RRE and Tat inducible (TAR) sequences. The instant specification (page 4, lines 7-18) acknowledges that Tat and Rev regulatory proteins allow efficient transcription and nuclear export of full-length vector transcripts, as described in Naldini (see p. 263, Col. 3).

However, Naldini does not disclose or suggest a hybrid intron comprising an SD and an SA site derived from different retroviral sources, as required by the claims. In addition, the coding sequence for the reporter gene lies outside of the RRE-containing intron (see Naldini, Figure 1).

Accordingly, Naldini et al. fails to cure the deficiencies of Lisziewicz. Naldini, either alone or in combination with Lisziewicz and/or Hope et al., does not teach or suggest the claimed invention.

In summary, none of the cited references teach or suggest the claimed invention. Removal of the obviousness rejection is requested.

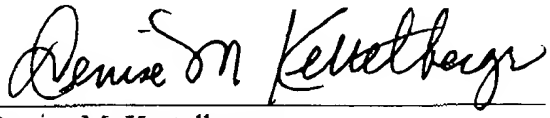
Conclusion

Applicants submit the claims are in condition for allowance. Notice of such allowance is requested. The Examiner is invited to telephone the undersigned attorney for clarification of any of the amendments and remarks or to otherwise speed prosecution of this application.

Applicants respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

MERCHANT & GOULD P.C.



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

24. (Amended) A retroviral vector particle comprising a packageable retroviral RNA genome ~~based on a first retrovirus~~ which, when in the form of a DNA provirus, ~~the RNA genome~~ comprises:

(i) a 5'LTR comprising ~~a~~ an HIV U3 and R region, or functional portions thereof having Tat inducible promoter activity ~~in place of the 5'LTR promoter function of the retrovirus on which the vector particle is based~~;

(ii) at least one nucleotide sequence (NS) capable of being expressed in a target cell; and

(iii) at least one retroviral polynucleotide response element (PRE) ~~derivable from a second retrovirus~~ which is responsive to a nucleus to cytoplasm transport factor;

~~wherein the NS is spliced and detectably expressed only in cells which also express Tat and Rev;~~ wherein the NS and the PRE are located within an intron in a transcription unit of the provirus~~;~~, wherein the intron is flanked by a retroviral splice donor (SD) site and a retroviral splice acceptor (SA) site~~;~~ derived from different retroviruses, wherein said NS is capable of expression in Tat and Rev expressing cells,~~;~~ and ~~wherein the SD site is derivable from the first retrovirus and the SA site is derivable from the second retrovirus~~ NS expression is undetectable in cells not expressing Tat and Rev genes.

25. The retroviral vector particle according to claim 24, wherein the polynucleotide response element is responsive to a transactivating retroviral nucleus to cytoplasm transport factor.

26. (Amended) The retroviral vector particle according to claim 25, wherein the polynucleotide response element is responsive to HIV Rev, or a functional equivalent thereof.

27. (Amended) The retroviral vector particle according to claim 24, wherein the polynucleotide response element is the Rev response element (RRE) from HIV, or a functional equivalent thereof.

28. (Amended) The retroviral vector particle according to claim 24, wherein the NS encodes a therapeutic gene.

29. (Amended) The retroviral vector particle according to claim 24, wherein the [first retrovirus is] packageable retroviral RNA genome is derived from an oncoretrovirus.

30. (Amended) The retroviral vector particle according to claim 29, wherein the oncoretrovirus is a murine leukemia virus (MLV).

31. (Amended) The retroviral vector particle according to claim 24, wherein the [second retrovirus is] retroviral response element is derived from a lentivirus.

32. (Amended) The retroviral vector particle according to claim 31, wherein the lentivirus is an HIV virus.

33. (Amended) The retroviral vector particle according to claim 24, wherein a packaging signal is contained within the intron in which the NS is located.

34. (Amended) A DNA construct encoding the packageable RNA genome for the retroviral vector particle according to claim 24 operably linked to a promoter.

35. The DNA construct according to claim 34, wherein the promoter is a strong promoter.

36. The DNA construct according to claim 35, wherein the promoter is a CMV promoter.

37. (Amended) The DNA construct according to claim 34, wherein the ~~selected gene~~ NS is absent and the construct ~~has~~ comprises an insertion site within the intron containing the PRE at which one or more NS may be inserted.

38. A retroviral vector particle production system comprising a host cell transfected with the DNA construct according to claim 34.

39. A retroviral vector particle production system comprising a set of nucleic acid sequences encoding the components of a retroviral vector particle according to claim 24.

40. (Amended) An *in vitro* method for infecting or transducing a target cell with a retroviral vector, ~~according to claim 24;~~ the method comprising:

(i) ~~providing the target cell;~~

~~(ii)~~ contacting the target cell with the retroviral vector according to claim 24; and

(iii) selecting for a target cell ~~which has been infected or transduced by the retroviral vector~~ that expresses the NS.

41. (Amended) ~~The~~ Target cells ~~resulting from~~ produced by the method ~~according to~~ of claim 40.

42. (New) The retroviral vector particle of claim 24, wherein the SA site is derived from HIV.

43. (New) The retroviral vector particle of claim 24, wherein the SD site is derived from MLV.